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Bio-Ethanol Production from Fruit and Vegetable Waste by Using *Saccharomyces Cerevisiae*

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Bioethanol can be produced by the fermentation of fruits and vegetables waste, which carried out in schott bottle using *Saccharomyces cerevisiae*. Objective of this experiment was to determine the bioethanol percentage from fruit and vegetable wastes produce through a fermentation process using the yeast, *Saccharomyces cerevisiae* and to analyze the chemical amount and glucose content in production of bioethanol. At the end of this experiment, the best wastes that led to the highest production of bioethanol were recoded. This experiment has begun with sample collection step, sample preparation step, following by transferring waste juice into 1L Schott bottle, fermentation of waste in incubator for 3 days at 30^{o}C and lastly end with sample analysis. From the experiment, it is proved that the bioethanol yield could be produced from fruits and vegetables wastes as the substrates. Besides, it can be concluded that the highest bioethanol yield could be produced using pineapple wastes and high concentration of elements were recorded in oranges bioethanol, more so glucose content was also recorded higher in orange waste.

Keywords: Bioethanol, fruits, vegetables, waste, *Saccharomyces cerevisae*

INTRODUCTION

The world is in urgent need to resort an alternative energy sources due to the rapid exhaustion of the world's energy supply (Ali and Khan, 2011). The inevitability of the oil depletion, global warming and the greenhouse effects put the earth on an alarming condition(Saifuddin et al., 2014).Besides, we can see the global are too dependent on limited-sources-petroleum-based fossil fuel that later cannot afford to meet the future demands. As depletion of world's fossil fuel happened, the prices of fuel continue rising up and the pressure for oil independence and environmental concerns creating a strong market for biofuels (Arapoglou et al., 2010). Moreover, the use of natural resources fuel had created vast side problems. The increasing of CO_2 level in surrounding are correlated with global warming that directly responsible by the negative result of burning petroleum-based fuel (Hossain et al., 2010). Global concern about climate change and the consequent need to diminish greenhouse gasses emissions have encouraged the use of bioethanol as a gasoline replacement or additive (GalbeandZacchi, 2002). Another problem arise was open dumping of waste which would give malignant to the nature habitat at surrounding area of the dumping sites.

An idea of producing energy in a solution form by utilizing the waste is easily adoptable, efficient and cheap. Recently, a renewable energy source including various biogases, liquid fuels, and solid biomass are the most rapidly growing renewable energy technologist (Chin and H'ng, 2013). Most of the previous studies reported that bioethanol used instead of fossil fuel can reduce the emission of greenhouse gases. Rajandran (2013) reported that life cycle, economic, environment and energy assessment provide policy for the use of energy. Mostly, any plants that are producing large amount of sugar used as raw materials to produce an ethanol. For instance, potato, sugarcane and pineapple are one of example of fruits and vegetables that produce high yield of bioethanol as byproduct due to high amount of sugar contain in it. Usually, bioethanol are produce in 3 ways which are firstly sugar directly convert into ethanol through fermentation process; next, starch are hydrolyze by specific enzyme into small molecules which is sugar and undergo fermentation process resulting in an ethanol solution as final product; lastly cellulose and hemicellulose was run into a specific process in order to produce ethanol production.

Use of biofuel has become very important due to depletion of fossil fuel and necessary to protect to environment from greenhouse emissions. Furthermore, exhaust gases of ethanol are much cleaner. It has been reported earlier that use of 85% ethanol and 15% gasoline reduced the emissions of greenhouse gases around 38% compared to fossil fuel. This study will give more knowledge on potential bio-ethanol that can be produce for scientific community and public to further studies regarding bio-ethanol production from wastes.Fruits and vegetables produce a significant amount of solid waste. Management of solid waste had become a greater concern because open dumping of solid waste is creating environment problem. From our online observation, research journal according to this topic mostly came from Africa and India. The bioethanol production from fruit and vegetable waste using *S. cerevisiae* less explored in Malaysia. The scientific community and public have less information about fruit and vegetable waste can produce bioethanol through fermentation technology.

Bioethanol is the most use biofuel which is an alternative source of fossil fuel has received special attention in global market (Singh et al., 2012).This study will give more knowledge on a potential of biofuel that can be produced for the scientific community and public to further studies regarding bioethanol production from wastes. The objectives of this research are to determine the bioethanol percentage from fruit and vegetable waste produce through a fermentation process using the yeast, *Saccharomyces cerevisiae* and to analyze the chemical amount and glucose content in production of bioethanol using various fruits waste.

MATERIALS AND METHODS

Raw materials

The raw material that was fruits waste (pineapple, orange, watermelon) and vegetables waste (potato, tomato, and other leafy vegetable wastes) that were brought from Giant Hypermarket and "PasarPagi Kuala Besut" and commercial dry yeast are collected from Institute of Biological Science, Faculty of Science, University of Malaya, 50603, Kuala Lumpur.

Collection of juice, fermentation and filtration

In order to produce bioethanol, two methods (chemical analysis and fermentation) were used. This project was focus on bioethanol production through fermentation process by using yeast, *S. cerevisiae*. The fruit and vegetable wastes were collected from the nearest market, needed to wash it first and chopped into smaller pieces together with the skin and were blended with the grinder.

After collection of juice from fruit and vegetable waste were poured in 1 L schott bottle. Fresh weight of collected juice was measured and recorded. Total soluble solids of collected juice were evaluated using hand refractometer before and after fermentation process according to the method described in Khandaker et al. (2012). The pH of each of fruit and vegetable wastes measured. The original pH for each wastes were listed as; potato at pH5-6, and agricultural waste fermented with *S. cerevisiae* at pH6 (Thippareddy and Agrawal, 2010). The pH for each of the wastes was adjusted from 5 to 6 by using 5 M sodium hydroxide (NaOH) to increase the pH and 1 M acid hydrochloride (HCl) to decrease the pH. *S.cerevisiae* (yeast) \ added to each of the Schott bottles that contained different fruits and vegetable waste and were shocked afterward. The fermentation was conducted in the incubator with temperature (30°C) for 3 days. After 3 days, the next step run for this sample after the samples were taken out from the incubator.

All the smaples were filtered by using filter cloth immediately after samples taken out from the incubator and poured into the cloth covered beaker. During the filtration samples were kept around two hours to make sure that no liquid in waste biomass. After the filtration raw ethanol yield (%), glucose content, pH and total soluble solids content were measured and recorded.

Determination of ethanol yield and reducing sugar content

The rawbioethanol yield was determined by the measurement of ethanol absorbance at 575 nm after conducting ethanol assay by Dicromate Colorimetric Method (William and Darwin, 1950) using spectrophotometer. The absorbance values were compared to the ethanol standard graph and the percentage of ethanol had been calculated. Glucose content was determined by DNS method (Miller, 1959) and compared to the absorbance taken from each samples to the standard curve of reducing sugar to calculate the sugar content. Reagent 3-5 dinitrosalicylic acid and a standard curve of glucose were used to measure glucose content in the samples before and after fermentation. The detail procedure was carried out according to the methods describe byMiller, (1959).

Measurement of metals composition in the bioethanol

Minerals aluminum (Al), calcium (Ca), iron (Fe), potassium (k), magnesium (Mg) and sodium (Na) content in the bioethanol produced from fruits and vegetables waste were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), whereas Inductively Coupled mass Spectrometry (ICP-MS) used for the measurement of zinc (Zn), chromium (Cr), Nikel (Ni), compper (Cu) and lead (Pb).

Statistical analysis:

The results in this experiment of triplicate were expressed as mean ± standard error. The data had been analysed using Microsoft Excel software. Statistical analysis was done using SPSS -22 software using one way ANOVA to determine the significance among the fruit and vegetable wastes and the value of $p > 0.05$ was considered as significant.

RESULTSAND DISCUSSION

pH of fruit and vegetable waste

The results showed that pH content of fruit and vegetable wasted decreased significantly before and after fermentation process. The pH value has significant influence on alcohol fermentation. The optimum pH range for *S.cerevisiae*used in fermentation from ethanol production is 4.0-5.0 (Zabed et al., 2014). However, it was reported that this most tolerant yeast could produce ethanol from potato at pH 5-6. While, the pH did not affect the ethanol's yield in the range of 3.5-6.0 when using pineapple effluent as substrates. The rate of bioethanol production from pineapple juice was the highest when the fermentation was carried out at lower pH around 3 to 4 (Hossain et al., 2006).

According to Figure 1, the pH of bioethanol produced from different wastes was determined. Based on this experiment, the initial pH of pineapple and cabbage (3.77 and 5.35) did not follow the optimum pH range. Gashaw (2014)stated that pH is one of the factors affecting ethanol yield. The final pH value of bioethanol obtained from waste was determined in pineapple at 3.82, orange (4.28), tomato at 3.79, potato at 4.62 and cabbage at 4.19 (Figure 1). It was observed that after fermentation pH was sharply drop in case of pineapple and orange waste. Another three types of vegetable waste pH also dropped down from 4.19 to 3.79 in tomato, 5.39 to 4.62 (Potato) and cabbage from 5.35 to 4.19. We noticed the drop of pH after fermentation because glucose converted to ethanol. Similar range of pH in fresh tomato was reported by Moneruzzaman et al (2009). The observed pH decreases could lead to diminution on enzymatic activity and low pH would inhibit the yeast multiplication (Tropea et al., 2014).

It could be observed from the results that pH value before fermentation that potato and cabbage had quite similar bar trend (5.39 and 5.35 respectively) while the mean value of tomato and orange were 4.19 and 4.18. The lowest value was in pineapple which had the pH value of 3.77 before fermentation. It could also be observed that after fermentation each waste was significantly different at $P<0.05$. After 72hours fermentation process, it could be observed that orange and pineapple were the lowestwith pH value of 2.28 and2.52 respectively. The highest pH value after fermentation was in potato which has the value of 4.62 (Figure 1).

Total Soluble Solids of fruit and vegetable waste

In the case of TSS, the TSS content had showed significant difference before and after fermentation for each fruit and vegetable waste (Figure 2). Fruit juice and TSS content are important parameters in industrial processing (Khandaker et al, 2013). From this experiment initial amount of TSS content for tomato, potato and cabbage recorded were 2.1, 3.1 and 2.7 respectively.But in fresh tomato fruits TSS content around 6 % Brix, which is higher than tomato waste (Moneruzzaman et al., 2008a). The highest decreasing of TSS content was at pineapple is which from 8.4 to 4.2 followed by orange waste from 16.3 to 11.8. The lowest decreasing of TSS content was in potato waste from 3.1 to 3.0 (Figure 2). It may be due to the utilization of sugar from the waste which corresponds to the biomass production of *S.cerevisiae*. The TSS content in cabbage was decreasing from 3.4 to 2.7 while TSS content for tomato both before and after fermentation did not changesignificantly (Figure 2).

At the end of fermentation TSS content of fruit and vegetable wastes decreased because sugar converted into ethanol after fermentation. It has been reported that TSS content of fruits and vegetables decreased at over ripen or senescence stage due to conversion of sugar into alcohol ().

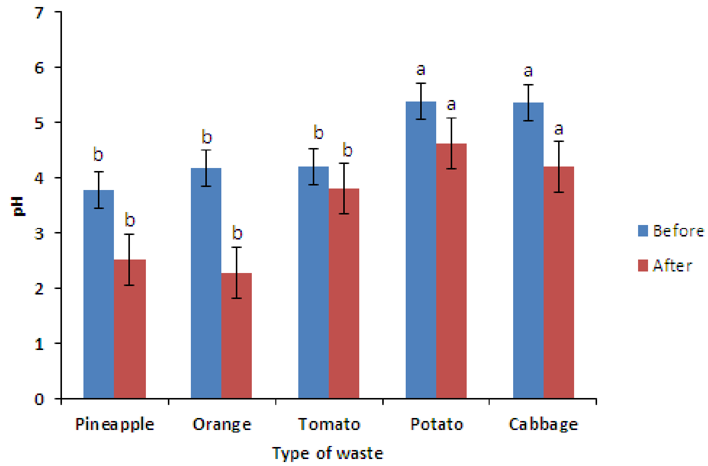

Figure 1:pH content of different wastes at before and after fermentation. Bars indicate ± SE.

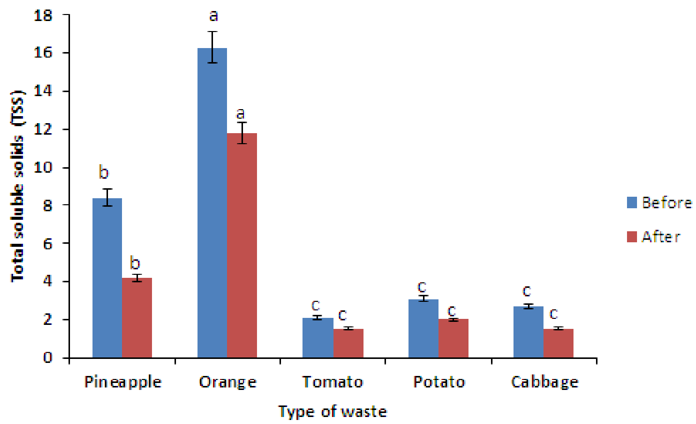

Figure 2:TSS pH content of different wastes at before and after fermentation. Bars indicate ± SE.

Bioethanol yield (%):

Bioethanol yield was investigated at different type of waste (Figure 3). The bioethanol yield at pineapple waste showed the highest with 5.371% followed by insignificant between tomato, 5.067% and orange 4.452%, cabbage 4.033% and the lowest is potato 0.929%. Agricultural wastes are rich source in fermentable sugars that can be good substrate for ethanol production, a potential energy source for crude oil (Itelima et al., 2013). It has been reported that ethanol from agricultural wastes can replace the gasoline in sustainable transportation fuel (Jimoh et al., 2009).Hence, the strain of yeast in *S. cerevisiae* has performed better in pineapple waste than the other fruits and vegetable wastes. In another study, bioethanol yield of from banana and orange peel were around 3 and 2% (Shilpa et al.,2013).

Kouakou et al. (2006) reported that ethanol concentration can be obtained at the range of 22.10 to 35.10 g/L at 25-46°C. In our study bioethanol production from potato was the lowest. The lowest production of bioethanol at potato can be improved by undergoing other methods before fermentation process which is starch hydrolysis, liquefaction and scarification process.

Glucose content at different incubation time

In this current study, pineapple and orange wastes produced the significant amount of bioethanol that's way we used these two samples for further analysis. From Figure 4, the reducing sugars measured were decreased as the fermentation was going on, and the bioethanol produced increased. From fermentation, the glucose had been utilized by yeast to produce bioethanol and carbon dioxide. Glucose content in orange dropped from 6.67 to 1.21mg after 120 hours of incubation, whereas glucose in pineapple starting from 4.53 at the beginning of incubation to 1.82mg at 72 hours and finally 1.21mg at 120 hours (Figure 4). Similar results was reported by Hossain et al. (2010), who reported that glucose content in pineapple waste decrease after incubation with east. It has been also reported that glucose content from the plant parts decreased with the storage time and temperature (Moneruzzaman et al., 2010).During plant growth and development, glucose content in the plant parts increased with phloemic stress (Saifuddin et al., 2009)

Metal content in bioethanol:

From the results, it can be seen that there are several additive metals presentin samples at concentration of yeast (1g/L)which had been plotted in the graphs as stated in Figures5 and 6. Additive metal consists of Zinc (Zn),Phosphorus (P), Calcium (Ca), Magnesium (Mg) and Boron (B). The concentration of yeast, oranges bioethanol got the highest values of concentration for all the major elements determined (Figure 5). Figure 5 also represent the content of Cuprum, Ferum (Fe), Argentum (Ag), Silicon (Sn), and Vanadium in the bioethanol from the orange wastes and all of this amount are significant compared to bioethanol from pineapple waste. Fe concentration in bioethanol from orange is 4.2 ppm.Contaminant metals Silicon (Si) and Sodium (Na) were also present in the bioethanol. An increasing linear trend of Si was also recorded. The results showed that orange waste produced significant amounts of elements than the pineapple waste. The Mn concentration of at both orange and pineapple bioethanol are minimal 5.3 and 4.5 ppm respectively. The results of this study showed that there were no dangerous elements in bioethanol from pineapple and orange wastes based on American Society of Testing and Materials (ASTM) and ASTM D5806. Zn and Ca content were found in the raw bioethanol from this two vegetable waste. These two compounds can change the alkaline reserve to neutralize acidic by-product of combustion and reduce the formation of insoluble compound and avoid corrosion to engine. Different additive elements like Zn, P, Ca, Mg and Boron were present in the bioethanol samples from pineapple and orange, and these elements may be from pineapple and orange wastes (Figure 5 and 6). Previous study reported that pineapple Juice consist of Pb, Mn, Ni, Zn and Sn trace elements (Williams et al., 2009).

It has been reported that mineral elements of pineapplesjuices consists of Na (2.24), K (124), Ca(11.5), Mg (15.4), P (3.16), Cu(0.059), Fe (0.265), Mn (0.295)and Zn (0.0074) Camara et al. (1994). Mineral concentration in fruit and vegetable juice varies with growing condition, growing medium, cultivar and harvesting maturity. Moneruzzaman et al.(2008b) reported that fruit physiological characteristics and quality of juice depend on the stages of fruit maturity. We found several elements in the bioethanol from pineapple and orange wastes that might be from the samples, or from the beaker, pH electrode and other equipment's using during the experiment.

Iron (Fe) and silicon (Si) elements may be come from sample waste or aluminum foil which is used during the sample preparation or fermentation.Aluminium foil consists of Al (51%), O (49%), Fe (0.95%), and Si (0.82%).The amount of Fe and Si exhibit in the surface of aluminum foil and can cause contamination during the use this foil. The pH electrode contains Ag CL_2 that can be precipitated inside the glass electrode.

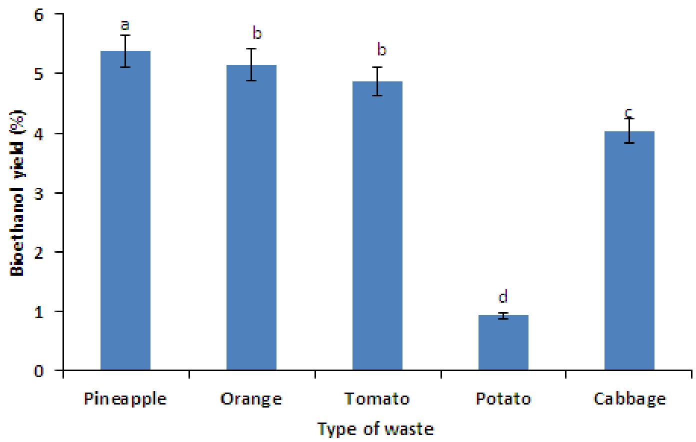

Figure 3:Bioethanol yield of different types of fruit and vegetable wastes. Bars indicate ± SE.

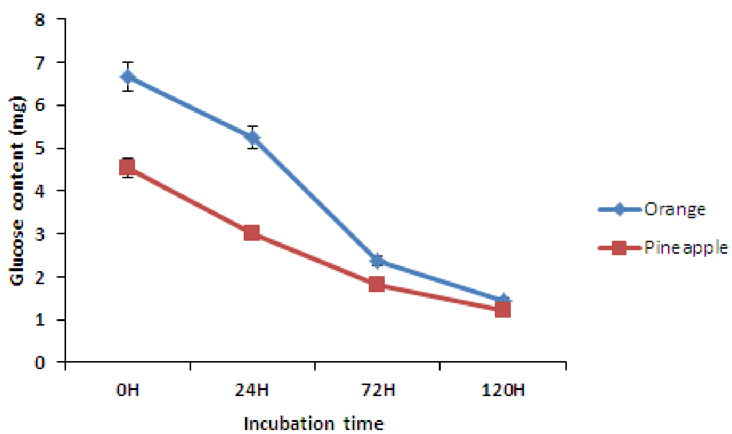

Figure 4: Glucose content (mg) measurement in different incubation times (hours). Mean ± S.E. are significantly different by ANOVA (P<0.05).

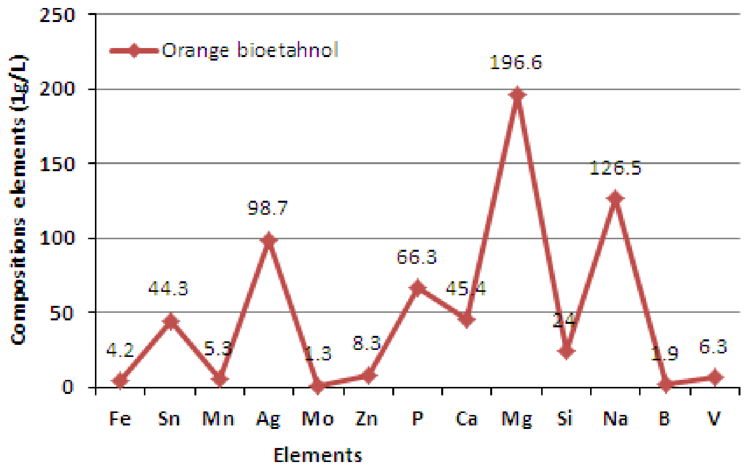

Figure 5: Compositions of elements of bioethanol from orange waste

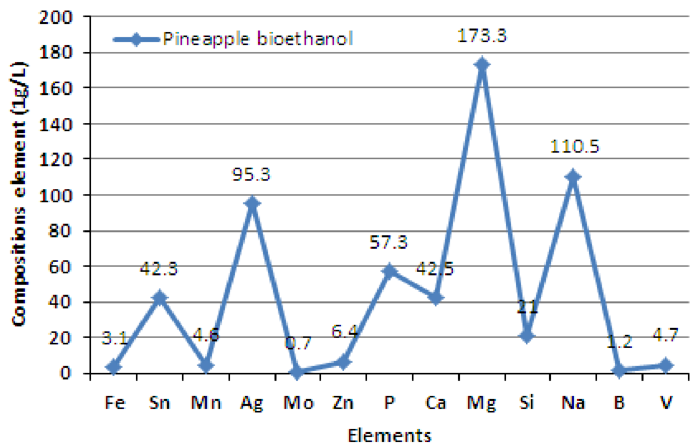

Figure 6: Compositions of elements of bioethanol from pineapple waste

It is believed that Ag in the sample bioethanol may be come from the pH electrode during pH measurement. Ag element can easily oxidize and mixed with the sample bioethanol. Sodium (Na) element in the sample may be come from the NaOH that was using in the fermentation form increasing sample pH. In this experiment borosilicate glassware were used and the borosilicate glass has nominal composition of SiO_2(0.70%), B (0.028), Na_2O (0.039%) and Al_2O_3 (0.01%)(Shrikhande and Gupta, 1998). NaOH can cause the corrosion of borosilicate glass (Manikandan et al., 1996).

They also recorded damaged glass surface by optical microscope. So, it can be sure that the presence of Si and B in the sample bioethanol from the corroded borosilicate glass not from the pineapple and orange wastes. The presence of Mo and V in the produced bioethanol from pine apple and orange wastes were believed to come from the samples. It might also be come from stainless steel apparatus, knives and spoon that were during the sample processing and fermentation. From the above discussion, it can summarized that the bioethanol produced from pineapple and orange wastes are safe to be used as one of the potential source of fuel, because no dangerous elements were not found and some elements presented in the sample bioethanol at the range of limit accepted on the standard of American Society of Testing and Materials.

CONCLUSION

In this study, results shows that different fruits and vegetables waste can serve as raw materials for the production of bioethanol, also it can be used as an alternative fuel to reduce the load on conventional fossil fuel resources. It is clear that the maximum yield of bioethanol was obtained from pineapple waste followed by orangewaste at temperature of 30˚C, high concentration of elements are recorded in orange waste.In addition, glucose content is also recorded higher in orange waste. The use of fruit wastes for bioethanol is cost effective and do not yields any toxic residues, however, optimization of substrate concentration and other environmental conditions are required for an industrial application.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MMK supervised and conducted the experiment.KBQ performed some part of the project. TLD wrote the paper. MHS, AM and ABMSH reviewed and edited the manuscript.

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